Molecular Models of Pyrethroid Metabolism by Carboxylesterases: Differential Effects Due To Stereochemistry

Melissa Pasquinelli, Rogelio Tornero-Velez*, James Rabinowitz

U.S EPA, ORD, Computational Toxicology Research Program

research&development

IIC-2

Science Question

Pyrethroids are a chemical class of widely used insecticides, and at least 16 chemicals in this class are registered for use in the U.S. Their mode of action in insects is to disrupt the sodium ion channels in nerve cell membranes and thus alter the normal function of the nervous system. These channels are also important target sites in mammals, but information on the effects of this class of chemicals on humans is limited. Experimental measurements of the hydrolysis of pyrethroids indicate differential rates of metabolism based on stereochemistry.

How do physical and chemical structure determine the role of pyrethroids in mechanisms of toxicity?

Research Goals

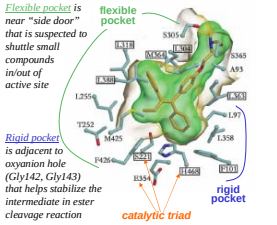
Utilize quantum chemistry and other molecular modeling methods in order to understand the relationship between molecular structure, metabolic rates and differential metabolism.

- Investigate the differential effects due to stereoselectivity for the metabolism of pyrethroids by carboxylesterase enzymes.
- Elucidate the mechanistic steps that need to be incorporated into physiologically-based pharmacokinetic (PBPK) models in order to extrapolate the knowledge from rodents to humans.

Background

Physiologically-based pharmacokinetic (PBPK) models of the chemical toxicity of pyrethroids are being developed by scientists in ORD/NERL. However, a limitation of PBPK models is the difficulty in obtaining experimentally-measured values for some parameters, especially for human models. Estimates for some parameters can be extracted from computational chemistry by either extrapolating from species to species or by calculating rates such as the metabolism of pyrethroids by an enzyme. Pyrethroids are metabolized by carboxylesterase enzymes that are found mainly in the liver. The catalytic triad residues (serine, histidine, and glutamic acid) cleave the ester bond in the pyrethroid molecules and form hydrolytic products.

Human CE1 with tamoxifen bound[1]

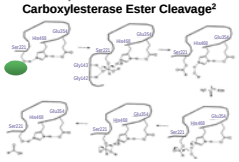


Flexible pocket is near "side door" that is suspected to shuttle small compounds in/out of active site

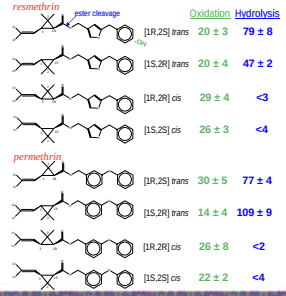
Rigid pocket is adjacent to oxyanion hole (Gly142, Gly143) that helps stabilize the intermediate in ester cleavage reaction

A similar catalytic triad exists in other enzymes such as serine proteases, so experimental and computational studies on the active site of these enzymes can be used to inform studies of the metabolism of pyrethroids by carboxylesterases.

Proposed Mechanism for Carboxylesterase Ester Cleavage[2]



Experiments on the metabolism of pyrethroids by carboxylesterases from rat reveal a dependence of the hydrolytic rate (ester cleavage) on the stereochemistry of the pyrethroid molecular structure[3].



	Oxidation	Hydrolysis
[1R,2S] *trans*	20 ± 3	79 ± 8
[1S,2R] *trans*	20 ± 4	47 ± 2
[1R,2R] *cis*	29 ± 4	<3
[1S,2S] *cis*	26 ± 3	<4
[1R,2S] *trans*	30 ± 5	77 ± 4
[1S,2R] *trans*	14 ± 4	109 ± 9
[1R,2R] *cis*	26 ± 8	<2
[1S,2S] *cis*	22 ± 2	<4

Approach

1. Prepare Protein Structure

Obtain protein crystal structure of the human carboxylesterase enzyme from the Protein Data Bank[4] (Entry 1MX1: Chains C, D). Prepare the protein structure for molecular modeling calculations[5]:

- Add missing atoms, including hydrogens
- Adjust the pKa of residues that potentially could be involved in interactions with the chemical agent

2. Prepare Pyrethroid Molecular Structures

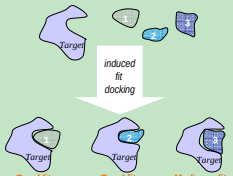
Crystal structures of two stereoisomers of permethrin exist in the Cambridge Crystallographic Database[6] (pojray, clvcpc01). Prepare each structure for molecular modeling calculations[5]:

- Add missing hydrogens and adjust the bond orders and charge states of the structure, if necessary.
- Create additional stereoisomers from these structures and perform a molecular mechanics minimization.
- Create resmethrin structures by modifying permethrin structure and perform a molecular mechanics minimization.

3. Dock Pyrethroid Molecular Structures Into Enzyme Active Site

Dock each stereoisomer of permethrin and resmethrin in the carboxylesterase protein crystal structure active site using induced fit docking[7] (1MX1: Chains C,D).

What is Induced Fit Docking?

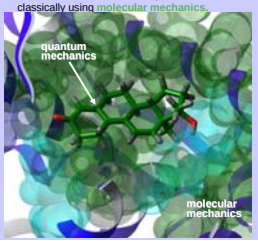


- Chemical agents are docked into the binding region of the macromolecular target.
- The structure of the chemical agent has torsional flexibility.
- The geometrical structure of the enzyme active site is flexible during the docking calculations so that the region can contort to properly fit chemical agents.

What is QM/MM?

Since the complex of a chemical agent and a macromolecular target is too large to be treated quantum mechanically, a mixed method is used.

- The physical-chemical properties of a chemical agent and any key surrounding atoms are described on a quantum mechanical level.
- The macromolecular environment that surrounds the chemical agent is described classically using molecular mechanics.



4. Optimize the Geometry of Each Pose of the Pyrethroid-Enzyme Complex with QM/MM

Optimize the geometry using QSite[8] of each docking pose obtained from the induced fit docking that has the pyrethroid ester group located near the catalytic triad of the carboxylesterase :

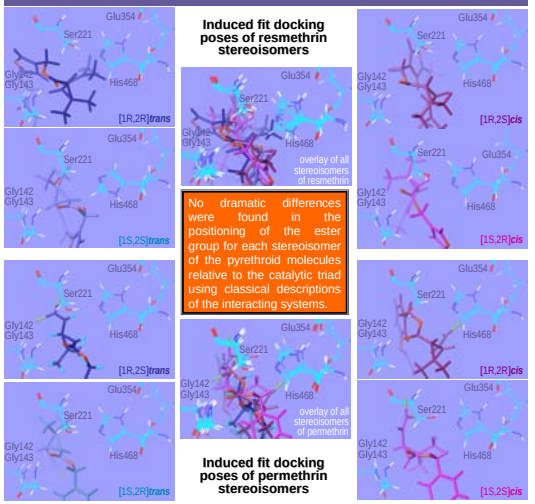
- **QM:** pyrethroid structure, residues of the catalytic triad, and possibly the two residues that form the oxyanion hole.
- **MM:** The rest of the enzyme structure. Constrain all residues beyond 8 Å from the pyrethroid, and freeze residues beyond 12 Å.
- Obtain chemical-physical properties such as the energy of the complex and the electronic charge distribution.

5. Calculate the Hydrolysis Rate of Each Pose of the Pyrethroid-Enzyme Complex with QM/MM

For each optimized geometrical pose, calculate using QSite[8] the differential metabolism rates along the ester cleavage reaction path (see proposed mechanism to the left); both the reactant and product geometries need to be defined for the reaction path method.

- **Reactant Geometry:** QM/MM optimized docking poses.
- **Product Geometry:** cleavage poses will be manually produced from the reactant geometry.
- *Assumption:* Rate limiting step is the ester cleavage or subsequent hydrolysis reaction.

Results



Title	Prime Energy	glide energy	Dist: S221(OG) lig(C9)	Dist: G142(H) lig(O3)	Dist: G143(H) lig(O3)	Dist: S221(HG) H468(NE2)	Dist: S221(OG) H468(NE2)	Dist: H468(HD1) E354(OE1)	Dist: H468(HD1) E354(OE2)	Dist: H468(HE2) lig(O4)	Dist: S221(OG) lig(O4)	Dist: S221(HG) lig(O4)
[1R,2R]trans res	-23961.0	-32.9	3.3	1.7	2.6	1.9	2.8	1.5	3.6	3.8	3.5	3.2
[1S,2S]trans res	-23956.8	-23.7	4.2	3.4	2.9	4.5	4.2	5.8	7.8	4.5	5.2	6.0
[1R,2S]cis res	-23955.2	-42.9	3.3	1.7	2.1	1.9	2.8	1.5	3.6	3.4	3.4	3.1
[1S,2R]cis res	-23963.1	-45.4	4.6	3.4	3.0	4.0	3.2	6.7	6.5	3.9	5.2	5.9
[1R,2S]trans perm	-23930.7	-48.8	3.3	2.1	3.4	1.9	2.9	1.5	3.5	4.5	4.3	4.2
[1S,2R]trans perm	-23932.6	-48.1	3.4	3.3	2.3	1.9	2.8	1.5	3.6	4.3	3.8	3.6
[1R,2R]cis perm	-23958.9	-44.8	3.1	2.2	2.0	4.6	4.2	1.5	3.6	3.3	3.3	4.1
[1S,2S]cis perm	-23898.3	-49.7	4.2	2.2	2.9	3.9	3.2	1.5	3.3	3.7	3.9	4.6

Future Directions

- Complete the calculations outlined in Steps 4 and 5 of the Approach Section.
- Repeat Steps 1-5 using the crystal structure of another species for which experimental metabolism data is available, such as rabbit.
- Run similar calculations on both the human and non-human species for other types of pyrethroids, in collaboration with scientists from ORD/NERL and ORD/NHEERL.
- Since these calculations are computationally rigorous, running these calculations for all pyrethroids is not feasible on the risk assessment timescale. However, a quantitative structure-activity relationship (QSAR) model can be developed from the information gained about a subset of chemicals. This QSAR model could interpolate the rates of metabolism of pyrethroids by carboxylesterases from rat or rabbit to human. This phase of the project could interface with the STAR Bioinformatics Research Centers.

Impact and Outcomes

This research supports the Agency's goals in the multi-year plans for Human Health and Cumulative Risk. It addresses the significant Agency need for predictive models for hazard identification in the sub-area (1) QSAR and other computational approaches.

This project supports two of the objectives of the NCCT as stated in the Computational Toxicology Framework: (1) improve understanding of the linkages from the source of a chemical release in the environment to the adverse outcomes, and (3) improve quantitative risk assessments. Molecular modeling can be used to not only explore the correlations along the toxicological pathway from exposure to biological effect, but it can also provide a means to include quantitative details into the risk assessment process.

In collaboration with Dr. Tornero-Velez in NERL and other ORD scientists, the use of molecular modeling approaches to probe metabolic and other toxicological mechanisms aids in the development of systems biology models. Molecular modeling is used to compute parameters for systems biology models that are not experimentally available, such as the rates of hydrolysis of pyrethroid chemicals by carboxylesterase enzymes. The knowledge gained from these calculations can also be utilized in order to develop predictive structure-activity relationships for the parameters of similar chemicals.

References

1. PDB entry 1YA4. C.D. Fleming, S. Benchariit, C.C. Edwards, J.L. Hyatt, L. Tsurkan, F. Bai, C. Fraga, C.L. Morton, E.L. Howard-Williams, P.M. Potter, M.R. Redinbo. *J. Mol. Biol.* 352, 165-177 (2005).
2. P. Buchwald, N. Bodor, *J. Med. Chem.* 42, 5160-5168 (1999).
3. D.M. Soderlund, J.E. Casida, *Pesticide Biochemistry and Physiology* 7, 391-401 (1977).
4. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne: The Protein Data Bank. *Nucleic Acids Research*, 28, 235-242 (2000).
5. Prime version 1.5, Schrödinger, LLC, New York, NY, 2005.
6. F.H.Allen, *Acta Cryst.* B58, 380-388, (2002).
7. Schrödinger Suite 2006 Induced Fit Docking protocol; Glide version 4.0, Schrödinger, LLC, New York, NY, 2005; Prime version 1.5, Schrödinger, LLC, New York, NY, 2005.
8. QSite version 4.0, Schrödinger, LLC, New York, NY, 2005.

* *Human Exposure and Atmospheric Sciences Division, National Exposure Research Laboratory, Office of Research and Development, U.S. EPA*

Long Term Goal II